Agric. Chem. Biotechnol. **44**(3), 147-153 (2001)

Fungicidal Activities of 51 Fruit-Derived Extracts *in vivo* against Six Phytopathogenic Fungi

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Received May 19, 2001

Methanol extracts from 51 fruits were tested for their fungicidal activities against six phytopathogenic fungi in a greenhouse. The efficacy varied with both the plant pathogen and fruit species used. At 10 and 5 mg/pot, methanol extracts of *Poncirus trifoliata* peel and seed gave over 80% control values against *Pyricularia grisea*, and strong fungicidal activities against *Rhizoctonia solani* were showed from the extracts of *Citrus paradisi* peel and *Punica granatum* leaf. In a test with *Botrytis cinerea* at 5 mg/pot, the extracts of *C. sinensis* seed and *D. kaki* leaf produced potent fungicidal activities, and the extracts of *C. crenata* peel and leaf, *Ch. sinensis* seed, *P. trifoliata* peel, and *Z. jujuba* leaf had strong fungicidal activities. At 5 mg/pot, strong fungicidal activities were produced in the extracts of *P. trifoliata* peel and seed against *Phytophthora infestans* and in the extracts of *P. ussriensis var. macrostipes* fruit and seed, *C. crenata* peel, *C. crenata* leaf, *C. paradisi* peel, *P. trifoliata* peel, *P. granatum* peel, and *Z. jujuba* leaf against *Puccinia recondita*. In a test with *E. graminis*, potent activities at 10 mg/pot were produced from the extracts of *Ch. sinensis* seed, *C. sinensis* seed, *P. trifoliata* leaf, *P. ussriensis var. macrostipes* fruit and seed, and *Vitis vinifera* seed. In the control effect of seven extracts against *B. cinerea* strains resistant to carbendazim, procymidone, and diethofencarb, extracts of *C. crenata* peel and leaf, *Ch. sinensis* seed, and *P. trifoliata* peel were highly effective against all strains of *B. cinerea*. Furthermore, potent fungicidal activities were produced from the extracts of *C. sinensis* seed and *D. kaki* leaf against the SSR, SRR, and RRS, and *Z. jujuba* leaf against the SSR and RRS strains. As a naturally occurring fungicide, these fruit-derived materials could be useful as new fungicidal products against phytopathogenic fungi.

Key words: *fungicidal activity, fruits, phytopathogenic fungi,* Botrytis cinerea.

The pre- and post-harvest losses due to fungal diseases in world crop production may be approximately 10.9 and 32.1%, respectively, or even higher in developing countries.[1,2] Over the past several decades, various attempts to control fungal diseases have taken an effort toward effective eradication or prevention through the development of synthetic fungicides. However, their continued uses cause many adverse effects such as human intoxication, environmental pollution,[3,4] resurgences,[5] resistance,[6,7] residue and toxicity to non-target organisms. Both economic consideration and increasing concern on adverse effects of the earlier types of fungicides have brought about the need for the development of alternative control methods with or without reduced use of organic fungicides.

Plants may provide an alternative to the fungicides currently used against fungal diseases, because they constitute a rich source of bioactive chemicals.[8,9] Since these are often active against a limited number of disease species, biodegradable to nontoxic products, and potentially suitable for use in the integrated management programs, they could lead to the development of new classes of possibly safer control agents. Therefore, much efforts have been focused on plant materials for potentially useful products as commercial insecticides or as lead compounds.[10-13] However, little work has been done on the fungicidal activities of various fruit extracts in spite of their excellent nutritional, pharmacological, and industrial significance.[14-17]

In the laboratory study described herein, we have examined extracts of various fruit for plant-derived fungicides against six phytopathogenic fungi (*Pyricularia grisea*, *Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora infestans*, *Puccinia recondita*, and *Erysiphe graminis*).

Materials and Methods

Plant materials and sample preparation. A total of 10 fruit species in 11 families were randomly and anecdotally collected (Table 1). They were dried in an oven at 60°C for 3 days and finely powdered using a blender (Model: RM 100, F.

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Abbreviations: PDA, potato dextrose agar.

Table 1. The yield of methanol extracts from 51 samples.

Scientific Name	Family Name	Part	Yield[a](%)
Actinidia arguta	Actinidiaceae	Fruit	2.8
Actinidia arguta	Actinidiaceae	Seed	5.1
Ananas bracteatus	Bromeliaceae	Fruit	5.9
Ananas bracteatus	Bromeliaceae	Leaf	4.3
Castanea crenata	Fagaceae	Fruit	2.4
Castanea crenata	Fagaceae	Peel	3.1
Castanea crenata	Fagaceae	Leaf	7.9
Chaenomeles sinensis	Rosaceae	Fruit	2.3
Chaenomeles sinensis	Rosaceae	Peel	2.9
Chaenomeles sinensis	Rosaceae	Seed	3.4
Citrus junos	Rutaceae	Fruit	9.6
Citrus junos	Rutaceae	Peel	4.3
Citrus junos	Rutaceae	Seed	5.3
Citrus paradisi	Rutaceae	Fruit	5.3
Citrus paradisi	Rutaceae	Peel	2.8
Citrus paradisi	Rutaceae	Seed	6.2
Citrus sinensis	Rutaceae	Fruit	2.6
Citrus sinensis	Rutaceae	Peel	5.1
Citrus sinensis	Rutaceae	Seed	3.5
Citrus unshiu	Rutaceae	Fruit	5.2
Citrus unshiu	Rutaceae	Peel	4.5
Citrus unshiu	Rutaceae	Leaf	4.9
Cucumis melo var. makuwa	Cucurbitaceae	Fruit	7.7
Cucumis melo var. makuwa	Cucurbitaceae	Peel	2.9
Cucumis melo var. makuwa	Cucurbitaceae	Seed	4.6
Cucumis melo Linne var. reticulatus	Cucurbitaceae	Fruit	6.5
Cucumis melo Linne var. reticulatus	Cucurbitaceae	Seed	7.4
Diospyros kaki	Ebenaceae	Fruit	4.2
Diospyros kaki	Ebenaceae	Seed	5.1
Diospyros kaki	Ebenaceae	Leaf	2.9
Fragaria ananassa	Rosaceae	Fruit	7.7
Malus pumila var. dulcissima	Rosaceae	Fruit	5.8
Malus pumila var. dulcissima	Rosaceae	Seed	6.2
Musa acuminata	Musaceae	Fruit	4.2
Musa acuminata	Musaceae	Peel	7.1
Poncirus trifoliata	Rutaceae	Fruit	2.5
Poncirus trifoliata	Rutaceae	Peel	1.9
Poncirus trifoliata	Rutaceae	Seed	2.3
Prunus persica	Rosaceae	Fruit	5.9
Prunus persica	Rosaceae	Seed	3.9
Punica granatum	Punicaceae	Fruit	2.5
Punica granatum	Punicaceae	Peel	3.2
Punica granatum	Punicaceae	Seed	3.0
Punica granatum	Punicaceae	Leaf	5.7
Pyrus ussriensis var. macrostipes	Rosaceae	Fruit	4.2
Pyrus ussriensis var. macrostipes	Rosaceae	Seed	3.6
Vitis vinifera	Vitaceae	Fruit	3.1
Vitis vinifera	Vitaceae	Peel	4.1
Vitis vinifera	Vitaceae	Seed	2.9
Zizyphus jujuba	Rhamnaceae	Fruit	2.6
Zizyphus jujuba	Rhamnaceae	Leaf	7.8

[a](Dried weight of methanol extract/dried weight of the sample fruit) × 100.

Kurt Retsch GmbH & Co. KG, Germany). Each sample (200 g) was extracted twice with 500 m*l* methanol at room temperature and filtered (Toyo filter paper No. 2, Toyo Roshi, Japan). The combined filtrate was concentrated *in vacuo* at 35°C using a rotary vacuum evaporator (Model: N-3NW, EYELA, Japan). The yields of 51 fruit extractions are shown in Table 1.

***In vivo* fungicidal activity test.** Six phytopathogenic fungi used in this study were *Pyricularia grisea*, *Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora infestans*, *Puccinia recondita*, and *Erysiphe graminis*. Except for *P. recondita* and *E. graminis*, which are not able to grow in artificial media, the others were routinely maintained on potato dextrose agar (PDA) slants and V-8 agar slants, and kept for stock at 4°C.

The fungicidal activities of test samples against pathogens used were determined using a whole plant spray method, as previously described.[18] Various fruit samples were tested at rates of 5 and 10 mg/pot. Test samples suspended in distilled water with Tween-20 added at the rate of 250 mg · ml^{-1} were used. Each test sample solution (50 m*l*) was sprayed onto two pots on the turntable at the same time. After evaporation in a greenhouse for 1 day, each pathogen was inoculated into the treated test plants. Untreated controls received Tween-20 solution. All treatments were replicated three times.

In a test with rice blast (RCB) caused by *P. grisea*, rice plants at the 2nd leaf stage (three plants/pot) were sprayed with each test solution. Treated plants were inoculated with suspension of conidia in distilled water (1×10^6 spores/m*l*) and kept in a chamber (25°C) for 24 h under 100% relative humidity (RH). Treated and control plants were then held in a lighted chamber (26 ± 2°C and 85% RH) for 5 days, and rated for the disease severity. For rice sheath blight (RSB) caused by *R. solani*, each test solution was sprayed onto rice plants at the 3nd leaf stage (three plants/pot). The plants were inoculated by injecting the inoculum at the base of the rice plants. Inoculum was made by culturing mycelial plugs in wheat bran medium at 25°C for 7 days, and macerated in a mixer. Treated and control plants were held in a lighted chamber (28°C) for 5 days. With cucumber gray mold (CGM) caused by *B. cinerea*, cucumber plants at the 1st leaf stage (one plants/pot) were sprayed with each test solution. The cucumber was inoculated with conidia (1×10^6 spores/m*l*) of B. cinerea incubated on PDA medium at 20°C for 15 days by leaf spray and then placed in a chamber (20°C) for 4-5 days. For tomato late blight (TLB) caused by *P. infestans*, each test solution was sprayed onto tomato plants at the 2nd leaf stage (two plants/pot). The plants were inoculated with a suspension of 1×10^5 zoosporangia/ml made from a 14-day culture of V-8 juice agar medium at 20°C. They were kept in a chamber (18°C) for 4 days, and disease ratings were made. For wheat leaf rust (WLR) caused by *P. recondita*, wheat plants at the 1st leaf stage (four plants/pot) were sprayed with each test solution. The plants were sprayed with a suspension (60 mg/100 m*l* of 250 ppm Tween 20°C) of uredospores collected from 2nd leaf of wheat, and then placed in a moist chamber. One day after inoculation, plants were held in a growth chamber (20°C and 70% RH). The fungicidal activities of the test samples were made 10 days after inoculation (DAI). For barley powdery mildew (BPM) caused by *E. graminis*, barley plants with fully expended first leaf (four plants/pot) were sprayed with a suspension of the test material. Treated plants were dusted with conidia of *E. graminis* collected from the primary leaf of barley and held in a chamber (20°C). The disease severity was rated on DAI.

The control effect of fruit extracts on each plant disease was evaluated with control value (CV) using the formula CV (%) = [(A − B)/A] × 100, where A and B represent the disease area on the untreated and treated plants, respectively. The responses were classified as previously described[18]: very strong activity ++++, CV >90%; strong +++, CV 81-90%; moderate ++, CV 61-80%; weak +, CV 40-60%; and little or no activity −, CV<40%.

Results

The fruit samples were randomly and anecdotally collected (Table 1). In this study, fungicidal activities of methanol extracts from 51 fruits in Actinidiaceae (2), Bromeliaceae (2), Cucurbitaceae (5), Ebenaceae (3), Fagaceae (3), Musaceae (2), Punicaceae (4), Rhamnaceae (2), Rosaceae (10), Rutaceae (15), and Vitaceae (3) were tested against six phytopathogenic fungi.

Fungicidal activities of the fruit samples against six plant pathogens are shown in Tables 2 and 3. The responses varied with fruit species and pathogens used. Methanol extracts of *Poncirus trifoliata* fruit, peel, and seed gave over 80% CV on *P. grisea* at a rate of 10 mg/pot. At 5 mg/pot, strong fungicidal activities were obtained from the extracts of *P. trifoliata* peel and seed, but extract of *P. trifoliata* fruit exhibited moderate activity (++). However, remaining samples (48 fruits) showed no activities against *P. grisea* when treated at 10 and 5 mg/pot. In a test with *R. solani*, strong fungicidal activities were produced from the extracts of *Citrus paradisi* peel and *Punica granatum* leaf at 10 mg/pot, and the extract of *P. granatum* leaf also exhibited potent activities at 5 mg/pot. However, other extracts showed weak or no activities against *R. solani* at 10 and 5 mg/pot.

In a test with *B. cinerea*, potent fungicidal activities (++++) at 10 mg/pot were produced from the extracts of *Castanea crenata* peel and leaf, *Chaenomeles sinensis* seed, *Citrus sinensis* seed, *Diospyros kaki* leaf, *P. trifoliata* peel, and *Zizyphus jujuba* leaf, and strong fungicidal activities (+++) were observed in the extracts of *Citrus junos* seed, *C. sinensis* fruit, and *P. granatum* peel. When treated at 5 mg/pot, extracts of *C. sinensis* seed and *D. kaki* leaf produced potent fungicidal activities (CV >90%), and extracts of *C. crenata* peel and leaf, *Ch. sinensis* seed, *P. trifoliata* peel, and *Z. jujuba* leaf had strong fungicidal activities (CV >80%). However, moderate activity was produced from the extracts of *C. junos* seed, *C. sinensis* fruit, and *P. granatum* peel, whereas weak or no fungicidal activities were observed in other vegetables. Against *P.*

Table 2. Controlling effects of fruit extracts on six plant pathogenic fungi at 10 mg/pot.

Sample Name	Plant Pathogenic Fungi[a]					
	RCB	RSB	CGB	TLB	WLR	BPM
A. arguta (F)[b]	-[b]	-	+	-	-	-
C. crenata (P)	-	-	++++	++	++++	+++
C. crenata (L)	-	-	++++	++	++++	-
Ch. sinensis (F)	-	+	-	-	-	-
Ch. sinensis (P)	-	-	-	-	++	-
Ch. sinensis (S)	-	-	++++	-	-	++++
C. junos (P)	-	-	-	-	-	+++
C. junos (S)	-	-	+++	-	-	++++
C. paradisi (P)	-	+++	-	-	++++	-
C. sinensis (P)	-	-	+	-	-	-
C. sinensis (S)	-	-	++++	-	-	-
C. unshiu (F)	-	-	++	-	-	-
C. unshiu (P)	-	-	++	-	-	-
D. kaki (F)	-	-	++	-	-	-
D. kaki (S)	-	-	-	-	-	+
D. kaki (L)	-	-	++++	-	-	-
M. pumila var. dulcissima (F)	-	+	++	-	-	+
M. acuminata (F)	-	-	++	-	-	-
M. acuminata (P)	-	-	++	-	-	+
P. trifoliata (F)	+++	-	++	-	++	-
P. trifoliata (P)	++++	-	++++	++++	++++	-
P. trifoliata (S)	++++	-	+++	++++	+++	-
P. persica (S)	-	-	-	-	+	-
P. granatum (F)	-	-	++	-	+++	-
P. granatum (P)	-	-	+++	-	++++	-
P. granatum (L)	-	++++	-	-	-	++++
P. ussriensis var. macrostipes (F)	-	-	-	-	++++	++++
P. ussriensis var. macrostipes (S)	-	-	-	-	++++	++++
V. vinifera (F)	-	+	-	-	-	-
V. vinifera (P)	-	-	++	-	-	-
V. vinifera (S)	-	-	-	-	-	++++
Z. jujuba (F)	-	-	++	-	-	-
Z. jujuba (L)	-	-	++++	++	++++	-

[a]RCB, *Pyricularia grisea*; RSB, *Rhizoctonia solani*; CGB, *Botrytis cinerea*; WLR, *Puccinia recondita*; BPM, *Erysiphe graminis*; and TLB, *Phytophthora infestans*.

[b]++++, >90%; +++, 80-90%; ++, 61-80%; +, 40-60%; and -, <40%.

infestans, extracts of *P. trifoliata* peel and seed showed potent fungicidal activities (++++) at 10 mg/pot, and, at 5 mg/pot, these fruits exhibited strong activities (+++). Remaining fruit extracts showed weak or no fungicidal activities against *P. infestans* at 5 mg/pot.

Of the 51 fruit extracts used, at 10 mg/pot, the extracts of C. crenata peel and leaf, *C. paradisi* peel, *P. trifoliata* peel, *P. granatum* peel, *Pyrus ussriensis var. macrostipes* fruit and seed, and *Z. jujuba* leaf produced potent fungicidal activities against *P. recondita*, and extracts of *P. trifoliata* seed and *P. granatum* fruit showed strong activities against *P. recondita*. At 5 mg/pot, extracts of *P. ussriensis var. macrostipes* fruit and seed also produced potent activity, and strong activities (CV >80%) were obtained from the extracts of *C. crenata* peel, *C. crenata* leaf, *C. paradisi* peel, *P. trifoliata* peel, *P. granatum* peel, and *Z. jujuba* leaf. In a test with *E. graminis*, when treated with 10 mg/pot, potent activities (CV >90%) were produced from the extracts of *Ch. sinensis* seed, *C. sinensis* seed, *P. trifoliata* leaf, *P. ussriensis var. macrostipes* fruit and seed, and *Vitis vinifera* seed (Table 2). However, remaining fruit extracts exhibited weak and no fungicidal activities (Tables 2 and 3).

Because of their excellent fungicidal activities, the control effect of seven test extracts against three *B. cinerea* strains resistant to carbendazim, procymidone, and diethofencarb were determined at a rate of 10 mg/pot (Table 4). Extracts of *C. crenata* peel and leaf, *Ch. sinensis* seed, and *P. trifoliata* peel were highly effective against all strains of *B. cinerea*. Fur-

Table 3. Controlling effects of fruit extracts on six plant pathogenic fungi at 5 mg/pot.

Sample Name	Plant Pathogenic Fungi[a]					
	RCB	RSB	CGB	TLB	WLR	BPM
C. crenata (P)	-[b]	-	+++	+	+++	++
C. crenata (L)	-	-	+++	++	+++	-
Ch. sinensis (S)	-	-	+++	-	-	+++
C. junos (P)	-	-	-	-	-	++
C. junos (S)	-	-	++	-	-	+++
C. paradisi (P)	-	++	-	-	+++	-
C. sinensis (F)	-	-	++	-	-	-
C. sinensis (S)	-	-	++++	-	-	-
D. kaki (L)	-	-	++++	-	-	-
M. pumila var. dulcissima (F)	-	-	+	-	-	-
P. trifoliata (F)	++	-	+	-	+	-
P. trifoliata (P)	++++	-	+++	+++	+++	-
P. trifoliata (S)	+++	-	++	+++	++	-
P. granatum (F)	-	-	+	-	++	-
P. granatum (P)	-	-	++	-	+++	-
P. granatum (L)	-	++++	-	-	-	+++
P. ussriensis var. macrostipes (F)	-	-	-	-	++++	++++
P. ussriensis var. macrostipes (S)	-	-	-	-	++++	+++
V. vinifera (S)	-	-	-	-	-	+++
Z. jujuba (L)	-	-	+++	+	+++	-

[a]RCB, *Pyricularia grisea*; CGB, *Botrytis cinerea*; WLR, *Puccinia recondita*; BPM, *Erysiphe graminis*; RSB, *Rhizoctonia solani* and TLB, *Phytophthora infestans*.
[b]++++, >90%; +++, 80-90%; ++, 61-80%; +, 40-60%; and -, <40%.

Table 4. Control effect of fruit extracts on susceptible and fungicide resistant strains of *Botrytis cinerea*, whole plant test[a].

Sample Name	Strains			
	2-18	P2	DJ-78	SDT-17
C. crenata (P)	++++	++++	++++	++++
C. crenata (L)	++++	++++	++++	++++
Ch. sinensis (S)	++++	++++	++++	+++
C. sinensis (S)	++++	++++	++++	-
D. kaki (L)	++++	++++	++++	-
P. trifoliata (P)	++++	++++	++++	++++
Z. jujuba (L)	++++	-	++++	-

[a]Exposed at 10 mg/pot.
2-18 (SSR), susceptible to both carbendazim and procymidone, but highly resistant to diethofencarb; P2 (SRR), susceptible to carbendazim, but highly resistant to both procymidone and diethofencarb; DJ-78 (RRS), highly resistant to carbendazim and procymidone, but susceptible to diethofencarb; and SDT-17 (RSR), highly resistant to both carbendazim and diethofencarb, but susceptible to procymidone.

thermore, potent fungicidal activity was produced from the extracts of *C. sinensis* seed and *D. kaki* leaf against the SSR, SRR, and RRS and *Z. jujuba* leaf against the SSR and RRS strains.

Discussion

Greenhouse studies with methanol extracts from 51 fruits belonging to the families Actinidiaceae, Bromeliaceae, Cucurbitaceae, Ebenaceae, Fagaceae, Musaceae, Punicaceae, Rhamnaceae, Rosaceae, Rutaceae, and Vitaceae revealed several the extracts exert potent fungicidal activities against the economically critical phytopathogenic fungi. Fungicidal activity varied with both the fruit species and pathogen tested. In a test with phytopathogenic fungi, *B. cinerea*, *P. recondita*, and *E. graminis* were inhibited more effectively by the application of methanol extracts of various fruits than *P. grisea*, *R. solani*, and *P. infestans*. Jacobson (1989) pointed out that the most promising botanicals as sources of novel plant-based pesticides at present and in the future are species of the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, and Canellaceae.[19] It has been also reported that Annonaceous plant species can be employed as safe, effective, economical, and environmentally friendly pesticides at home gardens, greenhouses, and ornamentals.[20] Various compounds including phenolics, terpenoids and alkaloids exist in plants.[8,9] These compounds jointly or independently contribute to the generation of biological activities. About 18,000 secondary plant metabolites have been chemically identified so far.[8] Since these plant-derived extracts and phytochemicals act on various types of disease complex, and may be applied to the

plant in the same manner as other agricultural chemicals, they are being considered as potential alternatives for synthetic fungicides,[20,21] or lead compounds for new classes of synthetic fungicides such as podoblastin produced from *Podophyllum peltatum*.[20,22] However, little information is available on the fungicidal activity of fruit extracts.

Our *in vivo* study revealed 16 fruit extracts with significant fungicidal activities (>80% CV) against *B. cinerea*, *P. recondita*, and *E. graminis*, although nearly most test samples except for *P. trifoliata* seed and fruit were ineffective against *P. grisea*, *R. solani*, and *P. infestans*. In particular, the strong activities of *C. crenata* peel and leaf, *C. paradisi* peel, *Ch. sinensis* seed, *C. junos* seed, *C. paradisi* peel, *C. sinensis* peel, *D. kaki* leaf, *P. trifoliata* peel and seed, *P. granatum* peel and leaf, *P. ussriensis var. macrostipes* fruit and seed, *V. vinifera* seed, and *Z. jujuba* leaf against the fungi tested confirm their superiority and usefulness as potent fungicides. These fruits provide new clues for managing the plant pathogens in field ecosystem, although their effects on non-target organisms or environment remain unknown.

Current control of plant diseases is primarily based on repeated or continued applications of conventional fungicides. However, their extensive use for decades has led to a widespread development of resistance.[6,7] Therefore, more emphasis must be placed on selective plant disease control agents for use in integrated management. Certain plant-derived materials were found to be highly effective against fungicide-resistant pathogens. For example, natural compounds such as cinnamaldehyde and salicylaldehyde are effective against four strains of *Fusarium sambucinum* resistant to thiabendazole.[23] As revealed through study, some fruit extracts were highly effective against the four resistant strains of *B. cinerea*, indicating that they could be useful as new fungicidal products against field populations of *B. cinerea*.

In conclusion, the fruit-derived materials might be useful for developing new types of fungicides, or biorational management agents for controlling different plant pathogens on crops at the same time, although their effects on natural enemies, fruit qualities, or environment have not yet been fully investigated.

Acknowledgments. This work was supported by the grant of Post-Doc. Program, Chonbuk National University (2001).

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